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ABSTRACT: The mechanism by which stimulated polymorphonuclear leukocytes and neutrophils (PMNs) damage pulmonary vascular endothelium was investigated.

The authors assessed the ability of unstimulated and mechanically stimulated PMNs to adhere to pulmonary endothelial cells and, thereby, alter pulmonary vascular permeability, measured as the pulmonary filtration coefficient (*K*) and haemodynamics. PMNs were stimulated by gentle agitation in a glass vial for 10 s.

Perfusing lungs with the stimulated PMNs (n=6) resulted in significant accumulation of PMNs within the lungs, assessed by myeloperoxidase levels, and elicited a 4-fold increase in *K* and a 2-fold increase in pulmonary vascular resistance as compared to lungs perfused with unstimulated cells (n=6). The increases in *K* were completely blocked by GF109203X, a protein kinase C inhibitor (n=6); however, GF109203X only partially attenuated the increase in vascular resistance and had little effect on the accumulation of stimulated PMNs. An agonist of protein kinase C, phorbol myristate acetate, elicited dose dependent increases in both K and pulmonary vascular resistance even in the absence of PMNs (n=6).

These findings indicate that the increases in pulmonary filtration coefficient and pulmonary vascular resistance induced by polymorphonuclear neutrophils result from endothelial cell injury mediated by activation of protein kinase C within the endothelial cells themselves.
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Acute respiratory distress syndrome (ARDS) is thought to be typical of pulmonary inflammation and involving activation of polymorphonuclear leukocytes and neutrophils (PMNs) [1]. In addition, it is known that PMNs are activated during cardiopulmonary bypass [2], and therefore, they may play a role in the pulmonary injury associated with the procedure [3, 4]. It was postulated that during bypass, mechanical shear stress stimulates PMNs to increase the availability of adhesion molecules on their surfaces. Consistent with this notion, mechanical agitation for 30 s on a tube mixer induced an increase in the proportion of PMNs bearing receptors for C3b (C3b-R), now classified as CD 11b/CD 18 (Mac 1) [5], thus enabling them to adhere more readily to vascular endothelial cells. Once bound, PMNs probably injure endothelial cells and increase pulmonary vascular resistance by releasing chemical mediators such as leukotrienes, oxygen radicals and/or elastase.

Alternatively or additionally, the endothelial cell injury caused by stimulated PMNs may result from an interaction between polymorphonuclear adhesion molecules and an endothelial cell signalling pathway mediating conversion of xanthine dehydrogenase (XD) to xanthine oxidase (XO) [6], in heart and lung, XO is only present in the endothelial cells lining the affected vasculature [7]. Increased endothelial XO would be expected to elevate intracellular levels of cytotoxic superoxide anions (O_2^-), which would in turn result in endothelial cell damage and increased pulmonary vascular permeability. On the other hand, recent reports indicate that activation of protein kinase (PK)C results in cytoskeletal protein phosphorylation in pulmonary arterial cell monolayer, and then results in endothelial cell contraction and resultant barrier dysfunction [8, 9]. In this study, the authors addressed the question of whether stimulated PMNs increase pulmonary vascular permeability and/or resistance and, if so, whether PKC-mediated intracellular signal transduction is involved in the process.

Materials and methods

Isolation of polymorphonuclear leukocytes and neutrophils

Human PMNs were isolated from heparinized blood obtained from a healthy adult who had no infectious foci and was taking no anti-inflammatory medications. Blood samples were layered over Polymorphprep (Nycomed Pharma., OSIO, Norway), centrifuged at $500 \times g$ for 35 min at room temperature, and the resultant band of PMNs was harvested using a Pasteur pipette. The PMN fraction was resuspended in modified Hanks′ balanced salt solution (HBSS; Sigma, St. Louis, MO, USA) lacking calcium, magnesium and bicarbonate, and centrifuged again at $400 \times g$ for 10 min. The cells were finally resuspended in

HBSS in order to restore normal osmolarity and then counted using an automated cell counter (Courter T-890; Coulter Electronics Inc., Tokyo, Japan).

Isolated rat lung preparation

Twenty-four adult male Sprague-Dawley rats (248.6± 32.1 g) were used in this study. Each animal was anesthetized with pentobarbital sodium (50 mg·kg, *i.p.*); a tracheotomy was then performed, and a 15-gauge luer stub adapter (Clay Adams, Parsippany, NJ, USA) was inserted into the trachea. The carotid artery was then catheterized (PE 50; Clay Adams), 500 units·kg^{-1} of heparin was injected, and the rats were exsanguinated. At that point, the authors made a sternum-splitting incision and opened the pericardium. The pulmonary artery was catheterized by way of the right ventricle using polyethylene tubing (PE 200; Clay Adams) connected to silastic tubing (outside diameter (OD) 4.65 mm, inside diameter (ID) 3.35 mm; Dow Corning, Midland, MI, USA). The aorta was ligated, and the left ventricle catheterized. The authors then flushed the lungs with 50 ml of saline until the effluent was clear, and the lungs had turned white. The venae cavae were then ligated, and the heart and lungs were removed *en bloc*.

After recording the weight of the preparation, which included the heart, lungs and catheters, it was placed in a Plexiglas box (B-345; Lustro Ware, Tokyo, Japan) where it was suspended from a counterbalancing bar attached to a force displacement strain gauge transducer (FT pick up TB-61 1T; Nihon Kohden, Tokyo, Japan). The trachea was connected to a compressed-air source so that the airway pressure could be continuously maintained at 2.0 cmH_2O. The pulmonary arterial and left ventricular catheters were connected to arterial and venous reservoirs, respectively. The reservoirs were immersed in a water bath and warmed to 37°C, saturated with mixed gas containing 30% O_2, 5% CO_2, and 65% N_2 and could be individually set at various heights to yield desired vascular pressures.

The lungs were kept in an isogravimetric state under zone 3 conditions (pulmonary arterial pressure > pulmonary venous pressure > alveolar pressure). The heart and lungs were perfused with Krebs-Henseleit buffer containing 6% bovine serum albumin (fraction V, Sigma), and a constant pressure-flow system was used throughout the experiments. Pulmonary venous and airway pressures (PPV and P_{aw}, respectively) were adjusted to 2.5 and 2.0 cmH_2O, respectively, and pulmonary arterial pressure (P_{pa}) was adjusted so that the lungs were neither gaining nor losing weight.

Experimental protocol

The objective of these experimental protocols was to determine whether stimulated PMNs increase pulmonary vascular permeability and resistance and, if so, whether PKC mediates the effect. To accomplish this, four series of experiments were carried out in four groups of isolated rat lungs. In the first series (unstimulated group; n=6), 125 µL of saline was injected into the pulmonary artery; ~30 min later, unstimulated PMNs were injected. In the second series (stimulated group, n=6), 125 µL of Dimethyl Sulfoxide (DMSO; Sigma), the vehicle for GF109203X (Sigma), an inhibitor of PKC, was injected; ~30 min later, the perfusate was changed to control saline in order to avoid affecting the PMNs, and stimulated PMNs were injected. The third series (GF group, n=6) was identical to the second except that 5 µM GF109203X was added to the DMSO vehicle. To directly assess the extent to which activation of PKC in endothelial cells increases the filtration coefficient, in the fourth series of experiments (phorbol myristate acetate (PMA) group; n=6), either 20 or 200 nM PMA (Sigma), a PKC agonist, was injected into the pulmonary artery.

The pulmonary filtration coefficient (K) was measured before and after each injection of vehicle and again 90 min after injection of the PMNs, the authors measured (K), an indicator of vascular permeability. To calculate pulmonary vascular resistance, perfusate flow rate (Q'_p), P_{pa} and double occlusion pressure (P_{do}), which reflects pulmonary capillary pressure [10], were all measured. As mentioned above, P_{pv} was set at 2.5 cmH_2O. Once these measurements had been obtained, the lungs were perfused with either stimulated or unstimulated PMNs at a final concentration of 250 cells·µL^{-1} of perfusate (except PMA group) for ~10 min. The lungs were then flushed with 50 mL of saline to washout intravascular PMNs that had not adhered to endothelial cells and then stored (except PMA group) at -80°C until myeloperoxidase (MPO) measurements were made.

Stimulation of PMNs was achieved by shaking them gently in a glass vial for 10 s at room temperature (20± 2°C). The PMN specimens were made up of 83.3±6.3% neutrophils; the remaining cells were monocytes.

Measurements

P_{pa}, P_{pv} and P_{aw} were continuously measured with pressure transducers (P23ID; Gould Inc., Santa Ana, CA, USA), and Q'_p was monitored using an electromagnetic flow meter (FF050 and MF-27; Nihon Kohden); these four parameters as well as lung weight were all continuously recorded on a polygraph (WT-687G; Nihon Kohden).

Calculation of the pulmonary filtration coefficient

K was calculated as previously described [11]. Briefly, arterial and venous pressures were simultaneously increased by 3 cmH_2O and the time-dependent increase in lung weight, which occurred in two phases, was measured. The standard interpretation of the weight gain curve is that there is an early gain due to increased vascular volume and slower gain resulting from continuous filtration. The increases in vascular volume were complete within 3 min; consequently, this component could be discriminated from the filtration component by plotting the log of the weight gain as a function of time [11, 12]. By using the method of least squares to fit a line to the later phase (data obtained from the last 7 min) and extrapolating back to time zero, the authors obtained the initial filtration rate. K was calculated by dividing the initial filtration rate by the applied microvascular pressure increment and normalizing to 1 g of wet lung weight.

Calculation of the pulmonary vascular resistances

The pulmonary arterial and venous resistances (Ra, Rv) and the pulmonary arterial and venous resistance ratio (Ra/Rv) were calculated as follows:

$$R_a = (P_{pa} - P_{do})/Q'_p \quad (1)$$

$$R_v = (P_{do} - P_{pv})/Q'_p \quad (2)$$

$$R_a/R_v = (P_{pa} - P_{do})/(P_{do} - P_{pv}) \quad (3)$$

Myeloperoxidase assay

As an index of PMN accumulation in the lungs, MPO was measured using a luminescence technique. The frozen rat lungs were thawed, a volume of 0.02% cethyltrimethyl ammonium bromide (CTAB; Wako Chemicals, Tokyo, Japan) equivalent to five times the lung weight was added, and the lungs were homogenized at 0°C. The homogenates were centrifuged at 12,000 $\times g$ and 4°C for 15 min in a high speed, refrigerated centrifuge (HITACHI 18PR-3; Hitachi, Tokyo, Japan). The pellets were rapidly frozen in liquid nitrogen, placed on ice, and then homogenized by sonication (UD201; Tomy Seiki, Tokyo, Japan) at 30 W for 30 s (30 times of 0.5 s burst plus 0.5 s stand). Freezing and sonication of the pellets were repeated seven times. The homogenates were centrifuged at 16,000 $\times g$ and 4°C for 15 min, and the supernatants were collected for assay. Initially, standard reagent (50 µM superoxide dismutase (SOD), 20 µL; 2 mM desferrioxamine, 20 µL; 50 mM KBr, 20 µL; 40 mM H_2O_2 25 µL; 0.2 M acetate-buffer, 1 mL; distilled water, 765 µL; and 0.2 mM 2-methyl-6-[p methoxyphenyl]-3, 7-dihydroimidazo[1,2-a] pyrazin-3-one (MCLA; Tokyo Kasei Organic Chemicals, Tokyo, Japan], 100 µL) was injected into a luminescence reader (BLR301; Aloca, Tokyo, Japan), and the basal luminescence was characterized for 3 min. The luminescences of the test specimens (50 µL of supernatant) were then assayed.

Statistical analysis

Data are expressed as mean±SD and were analysed by analysis of variance (ANOVA). Probability (p) values of<0.05 were accepted as significant.

Results

Pulmonary vascular permeability

Addition of vehicle or unstimulated PMNs had no significant effect on baseline values of K (table 1). On the other hand, after stimulated PMNs were injected, there was a 4-fold increase in K, suggesting that significant injury to the endothelium had occurred. The increase in K induced by stimulated PMNs was completely blocked by pretreatment with GF 109203X (fig. 1, table 1). In the PMA group, K was unaffected by the low dose of PMA (20 nM), but at the higher concentration (200 nM), PMA elicited a significant increase in K in the absence of PMNs (fig. 2, table 1).

Pulmonary vascular resistance

Ppa and Pdo were not significantly affected by any of the treatment protocols (table 2). However, upon injection of stimulated PMNs, there was a significant increase in pulmonary vascular resistance (Ra, Rv and total vascular resistance (Rt)) and a concomitant decrease in Q'p; effects that were completely blocked by GF 109203X-induced blockade of PKC. Conversely, activation of PKC by PMA dose dependently increased Ra, Rv and Rt and decreased Qp.

Table 1. – Effect of experimental protocols on the pulmonary filtration coefficient (K)

	K mg·cmH_2O^{-1}·min^{-1}·g^{-1}		
Treatment	Baseline	Vehicle	Experiment
Unstimulated PMNs	4.97±1.14	4.45±0.87	4.45±3.23
Stimulated PMNs	5.88±0.55	5.55±1.51	23.52±12.82*,‡
Stimulated PMNs + GF 109203X	4.48±1.69	4.41±1.79	4.90±1.46
200 nM PMA (no cells)	5.15±2.16	5.07±1.78	13.36±6.90**

Values are expressed as mean±SD. PMNs: polymorphonuclear leukocytes; PMA: phorbol myristate acetate. *: p<0.05 *versus* baseline of stimulated group; ‡: p<0.05 *versus* value after injection of PMNs (Experiment) in the unstimulated group; **: p<0.05 *versus* baseline and low dose PMA (not shown).

Myeloperoxidase assay

The MPO levels measured following injection of stimulated PMNs (44.82±24.53 Kcpm) were significantly higher than those seen following injection of unstimulated PMNs (5.86±6.73 Kcpm; fig. 3). Thus, it appears that stimulated PMNs accumulated in the lungs to significantly greater extent than unstimulated cells. When the correlation between vascular permeability and PMN accumulation was analysed in the groups injected with either unstimulated or stimulated PMNs, a significant association between K and MPO levels was found (fig. 4). The accumulation of PMNs was apparently not PKC-dependent since MPO level in the presence of GF109203X (31.93±11.98 Kcpm) remained significantly higher than in the unstimulated groups and were not significantly different from the stimulated group.

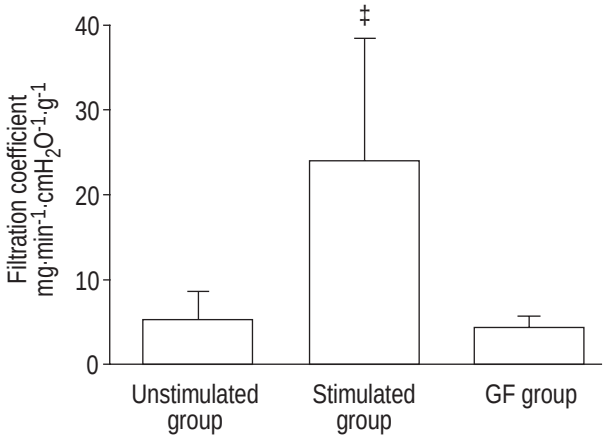

Fig. 1. – Effects of the protein kinase C inhibitor GF109203X (GF), on the increase in pulmonary vascular permeability (filtration coefficient) elicited by stimulated polymorphnuclear leukocytes and neutrophils (PMNs). Values are shown as mean±SD. ‡: p<0.05 *versus* unstimulated PMNs group.

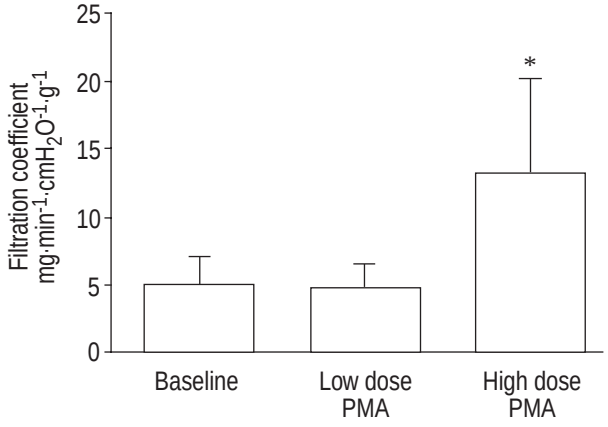

Fig. 2. – Effects of the protein kinase C agonist phorbol myristate acetate (PMA), on the increase in pulmonary vascular permeability (filtration coefficient). Final concentrations of low and high dose PMA were 20 and 200 nM, respectively. Values are expressed as mean±SD. *: p< 0.05 *versus* baseline.

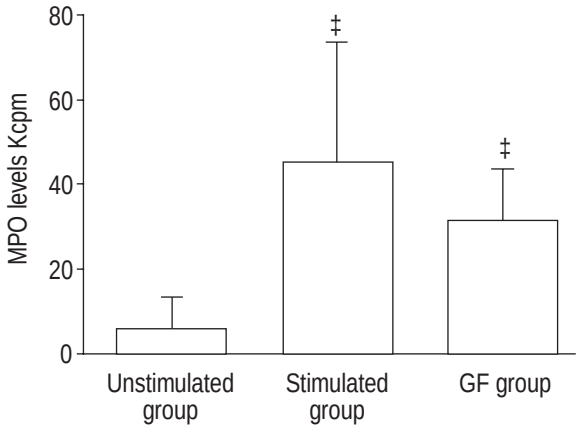

Fig. 3. – Myeloperoxidase (MPO) measured after injection of polymorphonuclear leukocytes and neutrophils (PMNs). MPO levels were higher in lungs injected with stimulated PMNs than in lungs injected with unstimulated PMNs. GF109203X (GF), a protein kinase C inhibitor, did not affect the elevated MPO levels induced by stimulating PMNs. ‡: p<0.05 *versus* unstimulated PMNs group.

Discussion

Little information is currently available on the mechanism by which stimulated PMNs evoke increases in pulmonary vascular permeability. It was found that stimulated PMNs significantly increased pulmonary vascular permeability after first adhering to the endothelial cells lining the pulmonary arteries. Consistent with this idea, CD 11b/CD18 (Mac 1) on the surfaces of PMNs were upregulated by mechanical stimulation [5], these β_2 leukocyte integrins are glycoprotein-derived adhesion molecules that enable PMNs to attach firmly to endothelial cells. PMN-induced increases in vascular permeability were blocked when stimulated PMNs were treated with anti-CD 18 monoclonal antibodies [5].

It was found that GF109203X, a PKC antagonist, blocked the PMN-induced increase in the vascular permeability, whereas PMA, a PKC agonist, increased vascular permeability even in the absence of PMNs. The dependence on PKC activation indicates that one or more intracellular signal transduction pathways must be involved. Vascular permeability has also been shown to be increased by superoxide anions, and the effect was blocked by allopurinol, a XO inhibitor [13–15]. It is postulate, therefore, that in heart and lung, the endothelial cell injury caused by stimulated PMNs results largely from an interaction between PMN adhesion molecules and an endothelial cell signalling pathway mediating conversion of XD to XO [6]. ln that regard, XO is only present in endothelial cell lining affected vasculature [7].

Intercellular adhesion molecule (ICAM)-1 is a member of the immunoglobulin superfamily; it has five immunoglobulin-like domains and is constitutively expressed on

Table 2. – Effect of experimental protocols on selected haemodynamic parameters

	P_{pa} cmH_2O	P_{do} cmH_2O	Q'_p $mL \cdot min^{-1}$	R_a $cmH_2O \cdot mL^{-1} \cdot min^{-1}$	R_v $cmH_2O \cdot mL^{-1} \cdot min^{-1}$	R_t $cmH_2O \cdot mL^{-1} \cdot min^{-1}$	R_a/R_v
Unstimulated PMNs							
baseline	11.4±1.1	6.1±0.5	14.8±4.7	0.39±0.13	0.27±0.12	0.66±0.24	1.53±0.50
vehicle	11.2±1.3	5.9±0.5	15.0±4.6	0.37±0.13	0.25±0.09	0.62±0.20	1.56±0.51
experiment	11.2±1.4	6.4±0.8	14.7±5.5	0.35±0.11	0.30±0.15	0.66±0.25	1.26±0.38
Stimulated PMNs							
baseline	9.9±0.7	5.3±0.5	11.3±4.1	0.44±0.14	0.30±0.18	0.74±0.30	1.71±0.65
vehicle	10.0±0.8	5.5±0.3	7.7±2.0	0.61±0.13	0.41±0.09	1.02±0.21	1.48±0.11
experiment	9.8±0.6	5.5±0.3	3.8±1.2**	1.21±0.38**,##	0.89±0.38**,#	2.09±0.75**,##	1.42±0.21
Stimulated PMNs + GF109203X pretreatment							
baseline	10.7±1.5	5.2±0.7	11.0±3.0	0.55±0.24	0.25±0.07	0.80±0.27	2.25±1.18
vehicle	11.1±1.1	6.0±0.5	8.7±2.1	0.60±0.15	0.42±0.12	1.03±0.24	1.49±0.46
experiment	11.1±0.8	6.4±0.8	7.3±1.6	0.68±0.27	0.56±0.18	1.24±0.42	1.23±0.31
PMA stimulation without PMNs							
baseline	9.8±0.9	5.1±0.5	10.0±1.4	0.49±0.21	0.26±0.05	0.75±0.20	1.97±1.01
low dose	9.9±1.0	5.8±0.7	6.8±1.6*	0.62±0.18	0.50±0.14	1.12±0.24	1.32±0.49
high dose	10.1±1.2	5.2±1.0	3.3±1.4*,†	1.98±1.41*,†	1.11±0.64*,†	3.09±1.80*,†	2.04±0.90

Values are expressed as mean±SD. P_{pa} and P_{do}: pulmonary arterial and double occlusion pressures, Q'_p: perfusate flow rate; R_a, R_v and R_t: pulmonary arterial, venous and total vascular resistances; PMNs: polymorphonuclear leukocytes; PMA: phorbol myristate acetate. *: p<0.05 *versus* baseline; **: p<0.01 *versus* baseline; #: p<0.05 *versus* vehicle; ##: p<0.01 *versus* vehicle; †: p<0.05 *versus* low dose (20 nM) group.

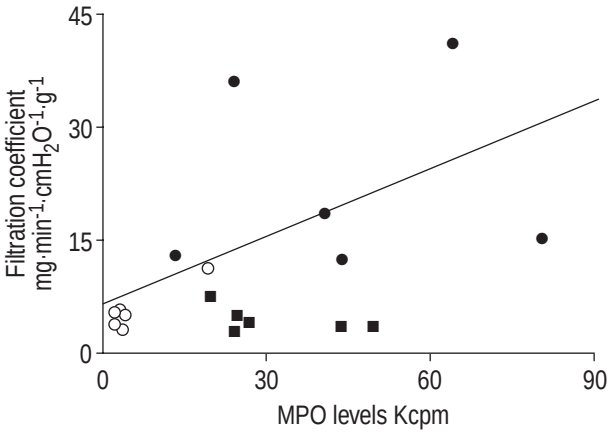

Fig. 4. – For each experimental group, pulmonary vascular filtration coefficients are plotted as a function of the myeloperoxidase (MPO) level. Each point represents a single experiment. The regression line was calculated using the data obtained from the stimulated (●) and unstimulated (○) groups (n=12; r=0.62, p<0.05). The data from the protein kinase C inhibitor FG109203X (GF) group (■) do not fit the regression line.

endothelial cells [16]. By interacting with ICAM-1 and leukocyte function associated antigen (LFA)-1 on the surface of endothelial cells, attached PMNs induce conversion of XD to XO [6, 17], and the resultant generation of superoxide anion might be damaging to the endothelial cells.

Cytokines, including interleukin (IL)-1, tumour necrosis factor (TNF)-α and interferon (IFN)-γ, upregulate ICAM-1 on the surface of endothelial cells; signals arising from IL-1 or TNF-α act at the nucleus inducing transcription and expression of ICAM-1 messenger ribonucleic acid (mRNA) [18]. Analogously, PMA-stimulated CD4+ T-lymphocytes induce ICAM-1 expression [19], suggesting T-cells may act *via* a similar pathway. In a B-cell lymphoma line, the interaction of ICAM-1 and LFA-1 elicit tyrosine hyperphosphorylation of a number of proteins including p53/$p56^{lyn}$ kinase [20]. Lyn kinase has been detected in various haematopoietic cells, including B-cells [21], neutrophils [22], and eosinophils [23] and once phosphorylated may act as a cytoplasmic protein tyrosine (PTK). Another possible PTK is $p60^{src}$ which has been detected in a rat cerebral microvessel endothelial cell line [24].

Activated PTK in turn activates phospholipase C (PLC) which hydrolyzes phosphatidylinositol 1,4,5 trisphosphate forming inositol 1,4,5 trisphosphate (IP3) and 1, 2-diacylglycerol (DAG), second messengers that respectively increase intracellular Ca^{2+} concentration and activate PKC. The PKC activation would be expected to result in increased conversion of XD to XO, phosphorylation of myosin light chain and actin rearrangement [25]. Conversely, inhibition of PTK blocks both upregulation of ICAM-1 by IL-1 or TNF-α, [26] and conversion of XD to XO [17].

Recently, CARDEN *et al.* [27] reported that intestinal ischaemia reperfusion activated circulating neutrophils to promote elastase-mediated lung injury. Neutrophil elastases, proteases and thrombin are a family of serine protease. When activated, neutrophils release several proteases, especially serine protease elastase. They have traditionally been considered to participate principally in the degradation of extracellular proteins [28]. However, they are also signalling molecules that regulate multiple cellular functions by activating specific receptors [29]. Besides thrombin, trypsin, plasmin, granzyme A, and cath-epsin G activate proteinase activated receptor (PAR)-1 [30, 31]. PAR-1, a member of G-protein-coupled rec-eptors (GPCRs), couples to several different G-proteins [29]. The principal mechanism is through Gαq proteins, resulting PLC, phosphatidyl inositol 1,4,5 trisphosphate hydrolysis. Formation of IP3 and DAG activate PKC.

It was observed that PMA elicited a concentration dependent increase in the pulmonary vascular permeability, confirming the involvement of endothelial PKC in the response. P_{do}, which reflects pulmonary capillary pressure [10], was unaffected by any of the experimental protocols, indicating that the increases in K elicited by activated PMNs and PMA were in fact due to changes in pulmonary vascular permeability (*i.e.* nonhaemodynamic oedema). It is known that PKC has important functions in the regulation of endothelial permeability and in the maintenance of endothelial integrity [32]. For instance, PKC phosphorylates cytoskeletal proteins in cultured bovine pulmonary artery endothelial cells resulting in barrier dysfunction [8]. GARCIA *et al.* [33] indicated that endothelial cell contraction, gap formation and barrier dysfunction occur *via* myosin light chain kinase (MLCK) dependent and independent mechanisms and are modulated by both PKC and cyclic adenosine monophosphate (cAMP)-dependent protein kinase A activities. In the current experiments, the concentration of PMA was relatively high, therefore, some other kinases, such as protein kinase A, might be involved in the mechanisms of the increase in K. So far, little information is available on the mechanisms by which activation of PKC and/or other kinases evoke barrier dysfunction of endothelial cells. There are several possibilities that induce dysfunction of endothelial cells by activation of PKC including; conversion of XO to XD [6, 17] that induces generation of superoxide anions [13–15]; and activation of MLCK that induces actin rearrangement [25]. Recent reports indicate that endothelial MLCK regulates neutrophils migration [34, 35]. It is speculated that PMNs adhesion to the endothelial cells induces; activation of an intracellular signal transduction system in endothelial cells and finally leads to gap formation of endothelial cells, and neutrophil migration through the gap [36].

PKC-induced contraction of pulmonary vascular smo-oth muscle was probably responsible for the concentration dependent increases in R_a and R_v and the decrease in Q'_p elicited by PMA. Usually contraction of the pulmonary vessels decreases vascular surface area and, therefore, filtration. This means that vascular permeability may have been underestimated in the present study. Injection of stimulated PMNs increased vascular resistance in both the stimulated group and in the GF group where activation of PKC was blocked, although the effect was only statistically significant in the former. Thus, stimulated PMNs probably increased vascular resistance in two ways: they occluded capillaries by adhering to their walls [37] and they elicited PKC activation leading to vascular smooth muscle contraction.

In the present study, effects on endothelium were not isolated from those on vascular smooth muscle. To address that question, examinations using cell culture systems will be necessary.

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